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Lupin flour as a wheat substitute in conventional and sourdough breadmaking: impact on bread physicochemical properties and volatile profile

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Abstract

Enhancing the nutritional profile of baked goods while addressing sustainability challenges means finding different sources of functional, sensory and nutritional ingredients. The aim of this study was to evaluate native lupin flour versus spontaneously fermented lupin flour as ingredient for wheat breadmaking. For that purpose, wheat flour was supplemented with 15–30 g/100 g lupin flour (LF15, LF30) or freeze-dried lupin sourdough (LS15, LS30) and dough and breads were assessed in comparison with wheat bread (control). Both lupin flour and lupin sourdough decreased dough stability, delayed the fermentation and lowered the pH. The incorporation of lupin flour increased the hardness of the crumb, except for when adding sourdough (15 g/100 g) that increased the bread expansion and enriched the volatile profile of bread. The analysis of the volatile compounds confirmed that lupin flour conferred fatty, green odor due to octanal, and when in the form of sourdough brought sour, and almond notes from acetic acid and benzaldehyde, respectively. Overall, lupin addition is a strategy to produce bread aligned with current trends towards sustainable and plant-based diets, particularly in the form of spontaneous type IV whole lupin sourdough up to 15 g/100 g wheat replacement.

Keywords Pulses · Dough fermentation · Mixing properties · Texture · Crumb color · Volatile compounds

Introduction

Among the ingredients of vegetable origin characterized by a high nutritional value, pulses stand out as an excellent source of protein, dietary fiber, vitamins, and minerals [1]. In this scenario, where the exploration of novel food ingredients plant-based is imperative, bread emerges as a suitable and effective vehicle for the enrichment of macronutrients and bioactive compounds [2]. In fact, under a nutritional standpoint, blending cereals and pulses can supply all the essential amino acids given that pulses are high in lysine but low in methionine, while cereals are high in methionine but low in lysine [3]. Nevertheless, some basic technological challenges, mainly related to gluten dilution with pulses proteins must be considered. For example, a volume decrease was described when chickpea replaced wheat in breadmaking [4]. Moreover, Turfani et al. [5] reported that blends incorporating green lentil flour at 6, 12 and 24 g/100 g diminished stability and strength compared to the wheat control, whereas the final breads with the highest substitution level presented reduced loaf volume.

Among the less explored pulses, lupin can play a major role in providing high nutritional quality ingredients for the growing global population in the next several decades [6]. In fact, lupin (*Lupinus* spp.) is a leguminous seed with high protein content (about 35 g/100 g DM) and around 40 g/100 g amount of fiber (DM) [7]. Lupin proteins are rich in bioactive peptides associated with health-related benefits, such as hypoglycemic, cholesterol-lowering, and anti-oxidative effect [1]. On the other hand, certain substances contained in seeds, such as alkaloids, anti-nutrients or enzyme-inhibitors may have an unwanted effect [1, 8].

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Although different varieties of lupin have been incorporated in bakery products, the most utilized are *Lupinus albus* spp. and *L. angustifolius* spp. due to their large production worldwide and low content of alkaloids [8].

Studies have shown that in general the substitution of wheat by lupin in bread-making remarkably improved the nutritional profile in terms of proteins and minerals [8, 9]. Furthermore, Villacrés et al. [10] reported the increase in protein, fat and fibers when debittered and fermented lupin flour replaced up to 20 g/100 g of wheat. Lee et al. [11] reported that lupin-enriched bread increased satiety and reduced energy intake. However, the substitution (from 5 to 30 g/100 g) of lupin flour negatively impacted the rheological behavior of doughs and the technological quality of the breads [12]. Specifically, dough stability decreased due to gluten dilution [12, 13], resulting in breads with reduced specific volume and loaf height, besides increased crumb hardness [9, 13]. Even using composite flours containing 20 g/100 g of lupin flours significantly reduces bread volume, leading to hard breads that show more dense crumbs with smaller gas cells appearance [14]. However, the bread-making optimization, particularly mixing and proofing time can make a significant impact on the physical quality of those composite lupin-wheat breads [15].

Spontaneous fermentation has long been a historical keystone of baking traditions to improve the sensory and functional quality of breads. This process has been also effective when using blends of wheat-legume in the formulation, increasing the protein digestibility, reducing antinutritional factors and, with strong impact on color taste and flavor of bread [16]. Specifically, it has been reported that the beany or grassy flavor of lupin breads could be reduced or modulated through LAB fermentation, which decreased the content of volatile compounds such as 1-penten-3-ol, hexanal and 1-hexanol [17].

Like for wheat and other legumes, sourdough fermentation could exploit lupin flour properties improving dough rheology by enhancing extensibility, reducing crumb hardness of breads, and enriching aroma through peculiar compounds [18, 19]. Some attempts have been reported to improve the functionality of the lupin flour by Bartkiene et al. [20] who conducted studies using strains previously isolated from spontaneous rye sourdough (*Pediococcus pentosaceus*, *Pediococcus acidilactici* and *Lactobacillus sakei*) to produce a lupin sourdough after incubation at 30 °C for 24 h. It was observed that lupin flour provided a favorable environment for the growth of lactic acid bacteria (LAB). They reported that fortifying breads with LAB inoculated fermented lupin flour up to 10 g/100 g of wheat flour significantly reduced the pH and increased the TTA of doughs and breads, enhanced specific volume of the final products and increased extensibility, porosity and hardness. On the other hand, the spontaneously fermented sourdough at 15 g/100 g replacement lowered the bread crumb texture properties, volume and crumb porosity and increased hardness if compared respectively to controlled fermented lupin and wheat bread.

However, although this study may represent a starting point for the evaluation of the spontaneous fermentation of lupin, it did not consider either the maturation process, nor its inclusion in breads as powder carrying a type IV sourdough. Type IV sourdough is traditionally produced by inoculating a mixture of flour and water with selected microbial cultures, followed by maintenance through backslopping similar to Type I [21]. Despite the intrinsic variability due to raw materials and processing conditions [22], spontaneous fermentation mimics traditional processes, promoting a diverse microbial ecosystem that enriches bread flavor and texture [16]. In addition, no research has been conducted using lupin flour to obtain a mature sourdough and evaluated the effectiveness of using spontaneously fermented whole lupin sourdough in powdered form to enrich bread at different levels. Therefore, the aim of this study was to assess the impact of type IV whole lupin sourdough used as wheat flour replacer on the quality characteristics of both dough and bread, to understand its suitability and potential applications in the breadmaking process.

Materials and methods

Materials

Canada Western Red Spring (CWRS) wheat flour (*Triticum aestivum L.*) was kindly provided by Cereals Canada (Winnipeg, Manitoba, Canada), whereas whole white sweet lupin (*Lupinus albus* L.) flour was donated by Lupin Platform Inc. (Calgary, Alberta, Canada). Instant dry baker's yeast (*Saccharomyces cerevisiae*) from Lallemand Inc. (Montreal, Quebec, Canada) and commercial salt were used. All the chemical reagents were analytical grade.

Methods

Proximate composition

The wheat flour and lupin flour were analyzed in triplicate to evaluate the proximate composition. Moisture for wheat and lupin flour was obtained using an Infrared Moisture Analyzer (Model IR-35, Denver Instrument, USA). AOAC Official Methods [23] and AACC International Methods [24] were used to quantify crude fats (AOAC Official Method, 2003.06), total ash (AACC International Method 08-01.01), and total protein (AOAC Official Method 992.23) with a conversion factor of 6.25 for lupin flour and 5.7 for wheat flour. In addition, total dietary fiber was calculated following AOAC Official Method (991.43). Wheat flour contained (expressed as DW)16.13 ± 0.14 g/100 g, 2.03 ± 0.43 g/100 g, 0.69 ± 0.01 g/100 g and 2.91 ± 0.37 g/100 g, for protein, fat, ash and dietary fiber, respectively. The lupin flour proximate composition (expressed as DW) was 37.33 ± 0.07 g/100 g, 16.40 ± 1.86 g/100 g, 4.10 ± 0.03 g/100 g and 8.33 ± 0.45 g/100 g, for protein, fat, ash and dietary fiber, respectively.

Sourdough preparation and characterization

Three batches of a naturally fermented type IV sourdough were prepared without the use of baker's yeast or other starter culture with a 300 dough yield, by mixing 100 g of lupin flour with 200 g of tap water, according to Rizzello et al. [16], with modifications. Briefly, the dough was incubated at 30°C for 16 h and stored at 4°C for the next 8 h, 50% of back slopping was refreshed and fermented as indicated before. The refreshments were conducted until the pH became stable for three consecutive days, which occurred after 7 days of back slopping. Then, the sourdough was freeze-dried and ground with a pestle and mortar, packed in plastic bags and stored at −18°C till further analysis.

The pH and the total titratable acidity (TTA) of the sourdoughs were monitored regularly throughout the fermentation process. The pH was measured potentiometrically (Accumet AB150, Fisher Scientific, Hampton, USA), in triplicate, using a penetration electrode. For the determination of TTA, one gram of sourdough was mixed with 10 g of water, and the slurry was titrated against 0.1 mol/L NaOH following the method described by Lazo-Vélez et al. [25].

The microorganism counts, calculated as log CFU/mL, were obtained by diluting 10 g of sourdough (0, 2, 4, 6, and 7 days) in 90 g of peptone water, inoculating 1 mL of each dilution in 3 M™ Petrifilm™ (3 M™, London, Ontario, Canada) for lactic acid bacteria (LAB), or for yeasts. The plates, in triplicate, were incubated at 30°C for 48 h for LAB and at 25°C for 48 h for yeasts.

Flour mixing properties

Micro-doughLAB (Perten Instruments, Sydney, Australia) device equipped with DLW software (version 1.2.1.234) was used for assessing the mixing properties of the flours following the Standard Flour Testing Method [24]. Briefly, the flour (based on 4 g) was mixed at constant temperature (30°C) and speed (63 rpm) for 20 min, and the torque was recorded. A torque of 100 mNm (500 BU) was set up as the target torque to calculate the water absorption. Water absorption (%, expressed based on 14 g/100 g moisture content), development time (min), stability (min), softening (mNm), mixing tolerance index (MTI, mNm) and quality number were extracted from the data obtained in DLW graphic.

Breadmaking process

Wheat breads were obtained with white flour and in the presence and absence of lupin sourdough. The basic recipe, flour basis, included: 1 g/100 g of salt and 1 g/100 g of active dry baker's yeast, besides the amount of water previously determined using the Micro-doughLAB. Wheat flour was replaced by 15 g/100 g (LF15) or 30 g/100 g (LF30) of lupin flour for the samples without sourdough, and by 15 g/100 g (LS15) or 30 g/100 g (LS30) of lupin freeze dried sourdough for the sourdough supplemented breads. All the ingredients were mixed for 3 min at low speed, followed by an additional 5 min at medium speed using a KitchenAid (KitchenAid Mississauga, Ontario, Canada) equipped with a dough hook. Doughs were divided (50 g), manually shaped and placed it in silicon pans of 12 individual molds of 7.4 × 4.8 × 0.9 cm. Proofing was carried out at 30 ± 2 °C in a fermentation chamber, recording the pH before and after fermentation and the TTA in the fermented doughs. The dough volume increase was recorded using a graduated cylinder along fermentation. The fermentation time for each dough was defined as the time needed to reach the 75% of the maximum dough volume, as reported Lazo-Vélez et al. [25]. Baking was conducted in electric convection oven (Blodgett Oven Company, South Burlington, VT, USA) at 170°C for ≃ 20 min. Two batches were performed for each recipe. Breads were kept packaged in plastic pouches till conducting the physical characterization. One bread was frozen at −20°C and further lyophilized for the analysis of the volatile compounds.

Breads characterization

The pH and TTA of breads were evaluated as described for the sourdoughs but using one gram of freeze-dried bread. Bread slice area was calculated in the slice cross-section (2D area). Slices (1-cm thick) were scanned (Epson Perfection V3, Suwa, Japan) at 1512 × 1275 pixels, 600 dpi, 24 bits. Image analysis was carried out using ImageJ software (Wayne Rasband and contributors, Nationals Institutes of Health, USA, version 1.54 g), following the procedures described by Espinosa-Ramírez et al. [26] with slight modifications. Data for 2D area (mm^2) and crumb porosity (%) were calculated.

For Texture Profile Analysis (TPA), the AACC 74-09 method [24] was used with slight modifications. After 24 h baking, a 2 × 1-cm crumb disc (diameter x thickness) from the center of the slice was used. TPA was conducted in six discs using a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, UK). The crumb disc was double compressed with a 36 mm diameter cylinder aluminum probe (P/36R model Stable Micro Systems, Surrey, UK) up to 50% of strain. A 5 g of trigger force was applied twice, with pre-test speed set to 1 mm/sec, test speed and post-test speed set to 5 mm/s, and a 5 s pause between tests. The parameters obtained were hardness (g), springiness, cohesiveness, chewiness, and resilience.

Crumb color was quantified in triplicates of each batch with a colorimeter (Konica Minolta CM-3500d, Tokyo, Japan) using a CIE$L^*a^*b^*$ scale. The color characteristics analyzed were L^* (lightness from black (0) to white (100)), a^* (positive and negative values are associated to red and green, respectively) and b^* (positive and negative values represented the yellowness and blueness, respectively).

Determination of volatile compounds of breads

Volatile compounds of the bread samples were extracted by 75 µm carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber (Supelco, Bellefonte, PA, USA) at 40 °C for 50 min. The compounds were desorbed and separated using gas chromatography/mass spectrometry (GC-MS) (Agilent Technologies Inc., Santa Clara, CA, USA) following the method described by Difonzo et al. [27]. The identification of the volatile compounds was carried out using Mass Spec Detector software (Agilent Technologies Santa Clara, CA, USA), with reference mass spectra of the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) and Wiley (John Wiley & Sons, New York, NY, USA) libraries. The volatile compounds identified were quantified by considering the peak area of 1-propanol as the internal standard reference. Analyses were performed in triplicate.

Statistical analysis

Statistical analysis was carried out using Statgraphics Centurion XVII software (Version 19.2, Virginia, USA). Multivariate analysis of variance (MANOVA) was utilized to detect significant variations among the various variables at a confidence level of 95%. Upon observing significant effects, Tukey's test was employed to ascertain honest significant differences (HSD).

Table 1 Characterization of type IV lupin sourdough along refreshments

Time (days)	pH	TTA (mL of NaOH/10 g)	LAB (log CFU/mL)	Yeasts (log CFU/mL)
0	5.2 ± 0.0 a	n.d.	n.d.	n.d.
2	4.2 ± 0.1 b	14.3 ± 2.1 a	3.3 ± 0.1 a	3.3 ± 0.0 a
4	4.0 ± 0.0 c	23.5 ± 1.4 b	6.8 ± 0.0 b	3.2 ± 0.0 a
6	4.0 ± 0.0 c	29.5 ± 0.7 c	8.8 ± 0.0 c	3.0 ± 0.1 a
7	4.0 ± 0.0 c	31.5 ± 2.1 c	9.2 ± 0.2 d	4.0 ± 0.0 b

TTA total titratable acidity, *LAB* lactic acid bacteria, *n.d.* not determined

Results and discussion

Lupin sourdough characterization

Sourdoughs were made with the lupin flour, which gave an initial pH of 5.2. The pH progressively decreased, with a major pH reduction (to 4.2) after the second refreshment, confirming the LAB adaptation (Table 1). After the fourth refreshment (day 4), the pH remained constant. Simultaneously, there was an increase of the total titratable acids, even beyond the fourth refreshment, which indicated the growth of LAB. In fact, microorganisms count showed an increase in LAB for 7 days and a slight increase in yeast, reaching respectively 9.2 log CFU/mL and 4 log CFU/mL. Data reported by Rizzello et al. [16] for chickpea, lentil and bean sourdough (respectively at 320, 210 and 200 DY) are in agreement with the results obtained for 300 DY lupin sourdough regarding pH, TTA and LAB count. Based on the parameters measured and the data reported, 7 days was chosen for preparing the sourdoughs.

Impact of lupin sourdough on flour mixing properties

The substitution of wheat flour by lupin sourdough (LS15 and LS30) significantly ($P<0.05$) increased the water absorption (WA, Table 2). The major impact was due to the presence of the sourdough and not the level of substitution. Conversely, the replacement of wheat flour by lupin flour did not affect the WA of the dough. Similar results were obtained with 30 g/100 g of chickpea sourdough [25] and other non-wheat sourdough [28, 29]. Therefore, results suggested that the metabolites released by LAB and yeast during the sourdough production were responsible for increasing the WA of the wheat flour. As Arendt et al. [19] suggests, this effect may result from the exopolysaccharides produced by LAB, which have water-binding properties that increase the hydration capacity of the flour matrix.

Compared to the control, the time taken for the dough to reach optimal development during mixing was not significantly affected either by lupin flour or by lupin sourdough substitution. This indicated that lupin flour did not consistently alter the resistance to deformation of wheat flour, even after sourdough fermentation. On the other hand, the ability of the dough to maintain its structure during mixing, reported as stability was significantly ($P<0.05$) reduced by the lupin presence as flour. Similar stability reduction was

Table 2 Effect of lupin flour (LF) or lupin sourdough (LS) on the mixing performance characteristics of wheat flour

	Control	LF15	LF30	LS15	LS30
WA (g/100 g)	65.62 ± 0.00 b	65.76 ± 0.00 c	65.50 ± 0.00 a	69.40 ± 0.03 d	69.85 ± 0.02 e
Development time (min)	4.0 ± 0.1 ab	4.0 ± 0.1 ab	5.3 ± 0.8 b	3.6 ± 0.2 a	4.7 ± 0.1 ab
Stability (min)	6.2 ± 0.5 c	2.9 ± 0.1 b	2.7 ± 0.1 b	1.9 ± 0.2 ab	1.6 ± 0.0 a
Softening (m Nm)	15.00 ± 0.71 a	16.00 ± 1.41 a	20.00 ± 2.12 a	36.00 ± 1.41 b	38.00 ± 0.71 b
MTI (m Nm)	3.00 ± 0.14 a	3.00 ± 0.28 a	4.00 ± 0.42 b	7.00 ± 0.28 c	8.00 ± 0.14 d
Quality number	60.80 ± 0.71 b	55.95 ± 1.41 b	55.60 ± 3.25 b	33.15 ± 0.49 a	30.20 ± 0.28 a

WA water absorption, *MTI* mixing tolerance index

Control refers to the bread made with wheat flour. The numbers following the sample code (LF or LS) indicate the level (g/100 g, flour basis) of wheat flour replacement. Different letters in the same row indicate significant differences at $P < 0.05$

obtained when replacing 10 g/100 g of lupin flour in common bread mix, suggesting that it may be primarily due to gluten dilution with lupin proteins [30]. Nevertheless, since no further decreased was observed in LS30, other factors might be considered as responsible for the dough weakening. This could open a point of further exploration to consider the impact of alkaloids and enzymes inhibitors on the dough rheology. The decrease in stability was accentuated with lupin sourdough, as previously found Villacrés et al. [10]. Probably, the increased proteolytic activity and the lower pH could further weaken the gluten network [19, 20]. In fact, significant ($P < 0.05$) differences on dough softening were only observed for LS15 and LS30 samples. It must be stressed that wheat flour replacement up to 15 g/100 g with lupin flour did not modify either the dough softening or the MTI, the latter being significantly modified only with a 30 g/100 g substitution. Therefore, despite the gluten dilution, the resulting dough was able to hold the structure during mixing. Conversely, lupin sourdough induced a significant increase in dough softening and MTI, likely due to lupin sourdough presence as observed by Bartkiene et al. [20] with different *lactobacilli.* It could be speculated that this result might derived from protein and fiber degradation during the fermentation, resulting in dough weakening. In fact, Boukid et al. [1] and Rizzello et al. [16] found that fermentation of legumes such as lupine can result in partial degradation of proteins and fibers, decreasing dough elasticity. In consequence, in terms of Quality Number, with higher values indicating better overall dough quality, samples with sourdough substitution had the lowest scores (LS15 and LS30).

Bread dough performance during fermentation

The fermentation kinetics were recorded besides the pH and the TTA (Table 3). The time needed to reach the optimal volume was significantly ($P < 0.05$) affected by lupin flour and sourdough. While the control showed the fastest fermentation with higher fermentation rate, whereas the slowest was observed for LF15 and LS30. Likely lupin flour slowed down the fermentation, but increasing the level of lupin flour did not induce further delays. In the case of the lupin sourdough the highest replacement tested further decreased the fermentation rate. To ensure optimum dough proofing, the fermentation time was adapted based on the maximum fermentation volume attained by each dough. All the doughs were fermented for the time needed to reach 75% of the maximum volume. Wheat dough had the shortest fermentation (61.50 min) whereas the longest one took place for LS30 (81.25 min). Those results might be associated with gluten dilution, or the weakening of the gluten network reducing its capacity of gas retention [1, 7]. Similarly, Turfani et al. [5] reported the alteration of dough viscoelasticity when wheat was replaced by lentil (from 10 to 24%). However, in the case of the sourdough LS30, the extended fermentation might be more connected to the interaction between sourdough metabolites and yeast activities [31]. The pH levels before fermentation were significantly ($P < 0.05$) different among the samples, with lower

Table 3 Effect of lupin flour (LF) or lupin sourdough (LS) on the wheat dough fermentation characteristics

	pH before fermentation	pH after fermentation	Fermentation rate (mL/min)	Maximum fermentation volume (mL)	Fermentation time (min)
Control	5.98 ± 0.05 e	5.47 ± 0.01 c	0.22 ± 0.00 c	16 ± 0.00 c	61.5 ± 2.12 a
LF15	5.59 ± 0.01 d	5.23 ± 0.09 b	0.13 ± 0.01 a	16 ± 0.00 c	79.5 ± 2.12 b
LF30	5.35 ± 0.02 c	5.13 ± 0.07 b	0.16 ± 0.00 b	19 ± 1.00 d	80.13 ± 1.59 b
LS15	4.55 ± 0.01 b	4.43 ± 0.04 a	0.16 ± 0.00 b	12 ± 0.00 b	75.5 ± 0.71 b
LS30	4.26 ± 0.00 a	4.24 ± 0.01 a	0.11 ± 0.01 a	10 ± 0.00 a	81.2 ± 1.77 b

Control refers to the bread made with wheat flour. The numbers following the sample code (LF or LS) indicate the level (g/100 g, flour basis) of wheat flour replacement. Different letters in the same column indicate significant differences at $P < 0.05$

values observed in LS15 and LS30 due to the substitution with the sourdough. As expected, the inclusion of freeze-dried lupin sourdough reduced the pH of the doughs, and the extent of the decrease was dependent on the substitution level. Nevertheless, lupin flour also significantly lowered the dough pH, and again the lowering was more significant at the higher substitution level. After the fermentation process, significant lower pH was found again in LS15 and LS30 but with no significant ($P<0.05$) differences between them. The reduction in pH during fermentation (∧ pH) in the presence of lupin flour was much lower, indicating that wheat flour substitution decreased the release of compounds responsible of lowering pH during fermentation, or the released ones, particularly degraded proteins, were different. That effect was even more noticeable in the presence of lupin sourdough, showing a pH decrease of 0.25 and 0.04 in LS15 and LS30, respectively.

Table 4 Comparison of the physicochemical characteristics (acidity, texture, color and crumb structure) of breads obtained from (control), lupin flour (LF) or lupin sourdough (LS)

	Control	LF15	LF30	LS15	LS30
pH	6.03 ± 0.01 e	5.84 ± 0.01 d	5.65 ± 0.01 c	4.66 ± 0.00 b	4.54 ± 0.01 a
TTA (mL of NaOH/10 g)	0.59 ± 0.06 a	0.86 ± 0.06 ab	1.71 ± 0.13 c	1.04 ± 0.06 b	2.43 ± 0.13 d
Texture profile analysis					
Hardness (g)	235 ± 17 a	639 ± 89 c	490 ± 86 b	230 ± 31 a	716 ± 97 c
Springiness	4.54 ± 0.43 b	2.62 ± 1.87 a	2.49 ± 1.69 a	4.48 ± 1.66 b	1.01 ± 0.02 a
Cohesiveness	0.89 ± 0.01 d	0.81 ± 0.02 b	0.80 ± 0.01 b	0.84 ± 0.02 c	0.76 ± 0.01 a
Chewiness (g)	911 ± 1 ab	1344 ± 1 b	860 ± 2 ab	917 ± 1 ab	581 ± 1 a
Resilience	0.52 ± 0.01 c	0.48 ± 0.02 b	0.47 ± 0.01 b	0.49 ± 0.02 b	0.42 ± 0.01 a
Color					
L*	61.69 ± 1.49 b	69.21 ± 0.72 b	63.91 ± 3.41 a	65.89 ± 1.12 ab	69.27 ± 0.35 b
a*	− 0.17 ± 0.17 a	1.36 ± 0.30 c	0.76 ± 0.10 b	0.48 ± 0.14 b	3.31 ± 0.26 d
b*	15.67 ± 3.98 a	25.37 ± 1.01 bc	23.03 ± 1.77 b	22.49 ± 1.11 b	29.95 ± 0.74 c
Bread slice image analysis					
Area (mm^2)	160.56 ± 1.90 c	145.71 ± 1.51 b	150.16 ± 1.70 bc	186.22 ± 7.40 d	125.15 ± 0.98 a
Porosity (pores/cm^2)	12.54 ± 0.49 a	12.64 ± 2.81 a	13.25 ± 3.03 a	10.67 ± 0.34 a	18.49 ± 1.54 a

Control refers to the bread made with wheat flour. The numbers following the sample code (LF or LS) indicate the level (g/100 g, flour basis) of wheat flour replacement. Total Titratable Acidity. Different letters in the same row indicate significant differences at $P<0.05$

Bread physicochemical characterization

Upon baking, breads containing lupin flour had significantly lower pH than the control, therefore acids present in lupin flour or generated during fermentation might be responsible for this pH decrease (Table 4). Breads containing lupin sourdough showed further pH reduction, as expected.

TTA was significantly ($P<0.05$) different in all breads. The inclusion of lupin flour conferred some acidity to the bread, which was more marked when increasing the replacement to 30 g/100 g wheat flour. Additional acidity was provided by the lupin sourdough, particularly at the highest substitution in LS30 (2.43 mL of NaOH/10 g). The organic acids produced during the fermentation led to an increase of the TTA of fermented lupin samples, compared to same substitution amount in the unfermented samples. Bartkiene et al. [20] reported the pH decrease and TTA increase when fermenting lupin by LAB, and that acidity was detected in the breads containing 10 g/100 g of that fermented lupin. Therefore, the acidity of lupin containing breads seems to be greatly associated to degradation compounds released from lupin including organic acids (e.g., lactic and acetic acids) produced by LAB, as well as aldehydes and alcohols generated through microbial fermentation and Maillard reactions [17, 32]. Rizzello et al. [16] also underlined the finding of lower pH in wheat-legume (chickpea, lentil and bean) 15 g/100 g substituted bread compared to baker's yeast alone breads, due to the lactic and acetic acid produced during the sourdough fermentation.

Texture profile analysis showed significant ($P<0.05$) differences in hardness, springiness, cohesiveness and resilience among all samples compared to the control. The substitution with lupin flour significantly ($P<0.05$) increased the hardness of the breads, likely due to the gluten dilution. Nevertheless, higher replacement with 30 g/100 g lupin led to breads softer than those with 15 g/100 g lupin, suggesting that the lupin constituents (protein, fiber, and lipids) might play an additional role. The 15 g/100 g of lupin sourdough substitution allowed to reduce the crumb hardness compared to its counterpart with lupin flour, reaching the hardness of the control. Sourdough fermentation appeared to mitigate the hardening effect of lupin flour at the 15 g/100 g substitution level. Previously, Villacrés et al. [10] also reported hardening effect in wheat flour breads when substituted with solid-state fermented lupin (up to 10 g/100 g). Likely, the hydrolytic action of the microbes on the proteins and fibers, allowed the additional expansion of the bread structure, leading to softer crumbs. However, excessive hydrolysis seems to have a dramatic effect on the crumb hardness, as occurred in LS30. In case of springiness, no significant ($P<0.05$) differences were found between LS15 and control but differed significantly between these two breads and the others (LS30, LF30 and LF15). Statistical analysis showed that lupin inclusion, as a flour or sourdough, significantly ($P < 0.05$) diminished the cohesiveness of samples, considered as the resistance to deformation, compared to control. Regarding the energy required to chew the bread (chewiness), lupin flour inclusion did not have a significant impact compared to the control. In the case of resilience, which is related to the ability of the bread to regain its original height after compression, lower values were found in LS30 with significant ($P < 0.05$) difference compared to the control which had the highest rates. The present results outcome the tendency of lupin flour to lower the springiness, the cohesiveness and the resilience and increase the chewiness of breads. The lupin sourdough did not impact this trend in case of cohesiveness, chewiness and resilience. Similar results were described by Cacak-Pietrzak et al. [34] in sourdough breads substituted with 10, 15, 20 and 25 g/100 g of legume flour, in particular with 25 g/100 g of yellow lupine flour and narrow-leaf lupine flour that dramatically decreased the springiness and cohesiveness of bread crumb. Authors attributed those effect to the reduced retention of carbon dioxide due to weaker gluten network structure. Fermentation could therefore have influenced changes in properties such as bread volume and particle size of lupin flour that Villacrés et al. [14] identified as correlated with textural properties. Decreasing in hardness, springiness, cohesiveness and resilience were also observed when wheat flour was substituted by different amounts (from 12.5 g/100 g to 50 g/100 g) of lupin flour (*Lupinus mutabilis*) in baker's yeast leavened breads [33].

Color showed no significant ($P < 0.05$) changes in crumb luminosity due to sourdough substitution with slightly lower values observed in LF 30 (Table 4). However, all samples exhibited higher redness (a^*) compared to control, with the highest values found in LS30 (3.12). All lupin-substituted samples resulted higher in yellowness (b^*). This phenomenon can be attributed to the presence of carotenoids with yellow-orange color in lupin flour [35].

The image analysis of the breads was carried out to evaluate the 2D area (mm^2) as indicative of the bread expansion and crumb porosity (%). While porosity was not significantly ($P < 0.05$) modified by lupin flour and lupin sourdough substitution, 2D area was significantly ($P < 0.05$) different. Wheat flour substitution by lupin led to smaller breads with slightly lower expansion (LF15 and LF30) compared to control. Doxastakis et al. [36] observed a negative correlation between loaf volume and water absorption in 5 g/100 g and 10 g/100 g lupin flour substituted samples. However, in the present study the water absorption was adapted, removing that source of variability. Thus, any volume reduction must be associated to the gluten dilution, although no significant impact was observed with 30 g/100 g replacement, likely due to the high protein content of the wheat flour used (16.13 g/100 g). The inclusion of lupin sourdough LS15 led to the highest 2D area (186.22 mm^2), but that effect was not observed with higher amount of sourdough. LS30 showed the lowest expansion, likely due to the weakening of gluten promoted by lupin flour, but also the sourdough metabolites. A higher sourdough concentration (30%) increases acidity, which besides the proteolytic action of the LAB, reduce the ability of the bread dough to expand during baking [16, 37]. Despite the impact on the area, no significant differences were observed in the porosity of the crumb, obtaining similar structure.

Volatile profile of breads

Twenty-one volatile compounds (Table 5) were considered as key contributors to flavor profile of the examined breads, distributed into 9 aldehydes, 4 alcohols, 1 carboxylic acid, 1 ketone, 2 furan compounds, 2 pyrazines and 2 esters. The volatile profile was influenced by the crucial events in the production of bread, such as the cooking procedure, the lupin flour fermentation and the addition of sourdough in the formulation, which partially replaced wheat flour. Overall, the volatile profile of the control bread showed significant differences compared to LF15 and LF30 breads. Although nine volatile compounds were detected in the control bread, their concentrations and diversity were lower than those observed in LF15 and LF30. This can be attributed to the absence of fermentation-derived metabolites such as pyrazines and esters, which are produced through microbial metabolism during sourdough fermentation. Lupin sourdough breads showed the highest amount of most of those compounds, and especially LS15 showed the richest profile. Moreover, certain volatile compounds (2-Heptenal, (E), Benzaldehyde, 2-Octenal, (E)-) were present exclusively in LS15 but not in LS30 bread, or their concentrations were doubled in LS15 compared to LS30. This result could be related to the combination of fermentation pathways enhanced by higher amounts of proteins, lipids and enzymes carried by lupin sourdough used as an ingredient. The amount of volatile compounds was lower in LS30, despite having sourdough concentration. Likely the increasing hydrolytic activity present in this bread, led to short fatty acids that were evaporated during baking.

Hexanal was the most represented volatile compound in all samples, giving a green-grassy and oily odor, followed by nonanal and benzaldehyde. A significant increase of nonanal, derived by the oxidation of oleic acid and also fermentative pathways occurring during dough fermentation and baking [38], was observed following the incorporation of lupin, proportional to the amount of lupin used. Indeed, *L. albus* has the highest content of oleic acid and MUFA

Table 5 Effect of lupin flour (LF) or lupin sourdough (LS) on the volatile compounds of wheat breads, considering wheat bread as control

Volatile compounds (μg/g)	Odor note[a]	Control	LF15	LF30	LS15	LS30
Aldehydes						
Hexanal	Green, grassy, tallow	380.12 ± 4.65 b	351.81 ± 21.15 b	407.02 ± 19.14 b	410.77 ± 16.42 b	400.82 ± 4.7 b
Heptanal	Fatty, green	0 ± 0 c	0 ± 0 c	0 ± 0 c	38.8 ± 15.51 a	2.21 ± 1.49 b
Octanal	Fatty, green	0 ± 0 c	0 ± 0 c	25.18 ± 3.16 b	56.23 ± 19.3 ab	35.09 ± 0.01 b
Nonanal	Green, fatty	68.58 ± 0.91 c	122.21 ± 13.33 b	212.23 ± 14.67 a	142.59 ± 17.23 b	257.49 ± 4.46 a
Decanal	Citrus	11.89 ± 1.94 b	0 ± 0 c	22.67 ± 0 a	0 ± 0 c	0 ± 0 c
2-Heptenal, (E)-	Green, fatty, fruity	19.77 ± 0.42 b	0 ± 0 b	20.96 ± 1.24 b	104.97 ± 35.7 a	0 ± 0 b
Benzaldehyde	Almond	16.19 ± 0.02 d	17.19 ± 0.71 d	31.9 ± 0.49 c	108.85 ± 37 a	56.82 ± 3.31 b
Furfural	Almond, burnt, roasted	5.12 ± 1.74 b	6.75 ± 0.62 b	6.1 ± 0.84 b	14.46 ± 3.24 a	11.48 ± 0.85 a
2-Octenal, (E)-	Fatty, green	0 ± 0 b	0 ± 0 b	0 ± 0 b	67.5 ± 23.21 a	0 ± 0 b
Alcohols						
1-Hexanol, 2-ethyl-	Fresh citrus	17.98 ± 1.91 b	0 ± 0 b	26.66 ± 8.45 b	31.67 ± 6.94 ab	27.61 ± 9.38 b
1-Octanol	Earthy and fat	0 ± 0 b	0 ± 0 b	50.85 ± 6.51 a	0 ± 0 b	0 ± 0 b
Phenylethyl alcohol	Rose-honey, wilted rose	0 ± 0 c	9.34 ± 2.34 bc	18.13 ± 1.85 b	33.72 ± 8.92 a	18.95 ± 1.39 b
1-Butanol	Sweet, balsam, fruit	0 ± 0 c	0 ± 0 c	6.52 ± 0.18 ab	8.94 ± 2.18 a	4.89 ± 0.09 b
Carboxylic acids						
Acetic acid	Sour, acid, pungent	3.2 ± 2.44 d	0 ± 0 e	13.83 ± 5.39 c	22.49 ± 13.51 b	48.94 ± 17.04 a
Ketones						
1-Octen-3-one	Artichokes, metal, mushroom	0 ± 0 b	0 ± 0 b	0 ± 0 b	12.44 ± 4 a	0 ± 0 b
Furan compounds						
Furan, 2-pentyl	Beany, fruity, green, earthy	9.33 ± 0 b	9.29 ± 0.68 b	17.45 ± 6 b	45.2 ± 15.94 a	16.94 ± 2.03 b
2-Furancarboxaldehyde, 5-methyl-	Almond, burnt sugar, caramel	0 ± 0 b	0 ± 0 b	0 ± 0 b	5.66 ± 2.21 a	0 ± 0 b
Pyrazines						
Pyrazine, 2-ethyl-6-methyl-	Roasted	0 ± 0 b	0 ± 0 b	0 ± 0 b	14.1 ± 4.61 a	0 ± 0 b
Pyrazine, 3-ethyl-2,5-dimethyl-	Baked, potato-like, earthy	0 ± 0 b	0 ± 0 b	0 ± 0 b	22.92 ± 4.95 a	0 ± 0 b
Esters						
Butanoic acid, octyl ester	Creamy, earthy	0 ± 0 c	0 ± 0 c	6.97 ± 0.72 a	4.27 ± 1.74 b	5.9 ± 0.02 ab
Acetic acid, phenylethyl ester	Jasmine, apple	0 ± 0 c	0 ± 0 c	0 ± 0 c	4.05 ± 1.08 b	20.89 ± 6.05 a

[a] Retrieved by FooDB Version 1.0, https://www.foodb.ca/. Control refers to the bread made with wheat flour. The numbers following the sample code (LF or LS) indicate the level (g/100 g, flour basis) of wheat flour replacement. Different letters in the same row indicate significant differences at $P<0.05$

among all lupin species (44–46% oleic acid and 51–54% of MUFA in *L. albus*; 28–36% oleic acid and 29–39% MUFA in *L. angustifolius*, and 20–24% oleic acid and 25–29% MUFA in *L. luteus*) [39] and, importantly, has more than double the oleic acid content of wheat flour, that amounts for about 16–20%. Conversely, the content of PUFA of *L. albus* = 29–31% (and in *L. angustifolius* = 35–49%, while in *L. luteus* = 53–59%), while in wheat flour accounts for 53–59% [40]. Higher presence of nonanal in LS samples was due to fermentative pathway activated by LAB and yeast metabolism during sourdough fermentation [18, 41], or the synergistic effect of lipid oxidation and microbial fermentation [42]. This balance between oxidative and fermentative pathways can be influenced by dough composition, fermentation rate, and pH.

Benzaldehyde, conferring a characteristic almond note, showed significant differences ($P<0.05$) in LF30, LS15 and LS30 compared to the control and LF15 with higher value in LS15 (108.85 µg/g). Fatty and green odors were supplied by nonanal, but also by 2-heptenal (*E*)-, heptanal and octanal, showing higher amounts in bread fortified with lupin sourdough. Decanal, another citrus-like volatile compound, was found in LF30 and control, showing significant differences ($P<0.05$) between samples and higher amounts in LF30 (22.67 µg/g). Decanal was recorded as a common volatile compound in breads with both sourdough and baker's yeast. Although, samples at lower percentage of lupin addition and/or with sourdough fermented lupin flour did not show this volatile compound. 2-Octenal (*E*)- was found only in LS15 (67.5 µg/g). This compound, together with the other mentioned aldehydes (e.g. hexanal and nonanal), and with 2-penthyl furan, was widely considered to be the main volatile compound responsible for the distinctive beany odor of pulses, included lupin, and derived food products Kaczmarska et al. [17]. Odors like almond, roasted or burnt were carried in all the samples by furfural, which displayed the maximum presence in LS15 with 14.46 µg/g and LS30 11.48 µg/g and statistical differences ($P<0.05$) between those two samples and control, LS15 and LS30. Furfural is a heterocyclic volatile compound originating during Maillard reaction, typically associated to roasted odors, and found in different cereal-based products Vurro et al. [43]. Except for nonanal, samples with 30% sourdough substitution showed reduced or absent aldehydes. This could be partially related to the attenuation of compounds typical of unfermented lupin or wheat, such as heptenal, (E)-2-heptenal, and furfural. The intermittent presence of other compounds could also be linked to variations in dough proofing times. Although unexpected, sourdough showed slight activity during proofing, suggesting a possible link between fermentation time and volatile compound differences, as reported by other authors [38, 41].

Among alcohols, the highest amount ($P<0.05$) of phenylethyl alcohol, with a characteristic honey-like and sweet fruity odor, was detected in LS15. This volatile compound was therefore more abundant when lupin sourdough was used, although it did not increase at higher percentage of flour substitution. 1-Octanol, instead, a volatile compound with an earthy fat-like odor found in the crumb and crust of lupin flour-enriched breads by other authors [44] was only detected in LF15 sample (50.85 µg/g). The absence of 1-octanol, and the reduction of phenylethyl alcohol, in 30 g/100 g sourdough-substituted breads could be due to increased fermentation of lupin flour, with the conversion of alcohols into the corresponding esters using Acetil-CoA [45].

In fact, higher contents of esters were found mainly in breads added of lupin flour at the highest percentage, especially fermented. Butanoic acid, octyl ester, known as creamy and earthy odor was detected in LF30, LS15 and LS30 showing the highest amount in LF30 (6.97 µg/g) with significant difference with LS15 ($P<0.05$) but without statistical differences with LS30. Jasmine and apple odor attributes were provided by acetic acid, phenylethyl ester. This compound was found only in sourdough-based samples, with significant differences ($P<0.05$) between LS15 (4.27 µg/g) and LS30 (20.89 µg/g), where the highest amount was shown. The reason why esters were found in sourdough-substituted samples or in 30 g/100 g lupin flour-substituted samples could be attributed to the combination of the higher protein and fat contributed by lupin flour and the baker's yeast and LAB activity, thus providing the substrates for the ester production [37].

Ketones were represented by 1-octen-3-one and were only found in LS15 sample. The combination of 1-octen-3-one (artichokes, metal and mushroom odors) and hexanal is typically associated to beany-like flavor of pulses and derived food products Bott and Chambers IV [46].

Regarding acetic acid, it was found in control, LF30, LS15 and LS30 exhibiting an increase of content the more lupin sourdough was added and with statistical differences ($P<0.05$) among all samples. Thus, a higher amount of this volatile compound providing a sour, acid and pungent odor was found in LS30 (48.94 µg/g) than, in decreasing order, in LS15, LF30 and control. A higher presence of acetic acid in the sourdough-substituted samples is also related to the liquid preparation of the lupin flour sourdough. The production of acetic acid is enhanced by yeast fermentation, as against the lactic acid production from LAB [47].

Finally, pyrazine compounds (2-ethyl-6-methyl-pyrazine and 3-ethyl-2,5-dimethyl-pyrazine) were found only in LS15. These Maillard reaction derivate compounds gave a typical roasted and baked odor, usually masking the beany flavor typical of pulses. The presence of pyrazines in few samples could be related to different pH, hydrolyzation level and amino acid-pyrazine specificity production in different samples due to the sourdough or flour substitution [32]. Considering their presence in sourdough samples, pyrazines could have been risen by the proteolytic release of amino acids and the amylolytic production of reducing sugars during fermentation. Moreover, the reaction could have been amplified by acidic environment and high baking temperatures [18, 39, 42].

In conclusion, the inclusion of lupin flour and especially its sourdough significantly changed and enriched the volatile profile of bread.

Conclusions

Lupin is a nitrogen-fixing species representing a sustainable, low water demanding and low carbon emitting non-dairy protein source. This study shows that lupin flour and sourdough influenced rheological properties of dough and bread quality. Lupin sourdough added at 15% improved the bread's expansion, the crumb structure and reduced hardness compared to lupin flour, on the other hand lupin flour and sourdough altered the dough mixing properties, varying water absorption, development time, stability, and mixing tolerance compared to wheat dough. Finally, the incorporation of lupin enhanced bread color, along with a richer volatile profile.

Overall, this study underscores the potential of lupin flour and lupin sourdough to improve the qualities of bread. In particular, the fermentation process using type IV sourdough unfolds some technological challenges firstly associated with gluten dilution However, the present study highlights that compounds present in the lupin flour dough, besides the hydrolytic activity of the LAB are responsible of the effects on dough and bread characteristics.

Furthermore, as the texture of bread presents slight changes, future research should focus on both consumer acceptability and optimization of fermentation conditions and sourdough formulation, to develop the benefits and broaden the application of lupin sourdough in the bakery industry.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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